

FIG. 1

1	5	10										
ATG	GCT	CCA	ATG	ACT	CAG	ACT	ACT	TCT	CTT	AAG	ACT	TCT
Met	Ala	Pro	Met	Thr	Gln	Thr	Thr	Ser	Leu	Lys	Thr	Ser
15								20				25
TGG	GTT	AAC	TGC	TCT	AAC	ATG	ATC	GAT	GAA	ATT	ATA	ACA
Trp	Val	Asn	Cys	Ser	Asn	Met	Ile	Asp	Glu	Ile	Ile	Thr
30									35			
CAC	TTA	AAG	CAG	CCA	CCT	TTG	CCT	TTG	CTG	GAC	TTC	AAC
His	Leu	Lys	Gln	Pro	Pro	Leu	Pro	Leu	Leu	Asp	Phe	Asn
40						45					50	
AAC	CTC	AAT	GGG	GAA	GAC	CAA	GAC	ATT	CTG	ATG	GAA	AAT
Asn	Leu	Asn	Gly	Glu	Asp	Gln	Asp	Ile	Leu	Met	Glu	Asn
55								60				
AAC	CTT	CGA	AGG	CCA	AAC	CTG	GAG	GCA	TTC	AAC	AGG	GCT
Asn	Leu	Arg	Arg	Pro	Asn	Leu	Glu	Ala	Phe	Asn	Arg	Ala
65						70				75		
GTC	AAG	AGT	TTA	CAG	AAT	GCA	TCA	GCA	ATT	GAG	AGC	ATT
Val	Lys	Ser	Leu	Gln	Asn	Ala	Ser	Ala	Ile	Glu	Ser	Ile
80							85				90	
CTT	AAA	AAT	CTC	CTG	CCA	TGT	CTG	CCC	CTG	GCC	ACG	GCC
Leu	Lys	Asn	Leu	Leu	Pro	Cys	Leu	Pro	Leu	Ala	Thr	Ala
95								100				
GCA	CCC	ACG	CGA	CAT	CCA	ATC	CAT	ATC	AAG	GAC	GGT	GAC
Ala	Pro	Thr	Arg	His	Pro	Ile	His	Ile	Lys	Asp	Gly	Asp
105							110				115	
TGG	AAT	GAA	TTC	CGT	CGT	AAA	CTG	ACC	TTC	TAT	CTG	AAA
Trp	Asn	Glu	Phe	Arg	Arg	Lys	Leu	Thr	Phe	Tyr	Leu	Lys
120								125				
ACC	TTG	GAG	AAC	GCG	CAG	GCT	CAA	CAG	ACC	ACT	CTG	TCG
Thr	Leu	Glu	Asn	Ala	Gln	Ala	Gln	Gln	Thr	Thr	Leu	Ser
130												
CTA	GCG	ATC	TTT	TAA	TAA							(SEQ ID NO: 144)
Leu	Ala	Ile	Phe	END	END							(SEQ ID NO: 138)

aa20 1 ATCGATGAAATCATCACCCACCTGAAGCAGCCACCGCTGCCGCTGCTGGACTTCAACAAAC
1 IleAspGluIleThrHisLeuLysGlnProProLeuProLeuAspPheAsnAsn -

60 E X h o I
C o R V
O R V
V R V
CTCAATGGTGAAGACCAAGATATCCTGATGGAAAATAACCTTCGTCGTCACCTCGAG
61 LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu -

120 P N
s s
t i
I I
GCATCAACCGTGCTGCTAAAGTCCTGCAGAATGCAT [SEQ ID NO:145] aa70
121 157 AlaPheAsnArgAlaValLysSerLeuGlnAsnAla [SEQ ID NO:146]

Clal to NsII Replacement Fragment

F I g - 2

N H
 C p
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 I I
 CCATGGCTCCAATGACTCAGACTACTTCTCTTAAGACTTCTGGGTTAACTGCTCTAAC
 1 -----+-----+-----+-----+-----+-----+-----+ 60
 GGTACCGAGGTTACTGAGTCTGATGAAGAGAATTCTGAAGAACCCAATTGACGAGATTGT
 MetAlaProMetThrGlnThrThrSerLeuLysThrSerTrpValAsnCysSerAsnMet

 C
 l
 a
 I
 TGATCGATGAAATTATAACACACTAAAGCAGCCACCTTGCCTTGCTGGACTTCAACA
 61 -----+-----+-----+-----+-----+-----+-----+ 120
 ACTAGCTACTTTAATATTGTGTGAATTCTCGTCGGTGGAAACGGAAACGACCTGAAGTTGT
 IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn

 ACCTCAATGGGAAGACCAAGACATTCTGATGGAAAATAACCTTCGAAGGCCAACCTGG
 121 -----+-----+-----+-----+-----+-----+-----+ 180
 TGGAGTTACCCCTCTGGTTCTGTAAGACTACCTTTATTGGAAGCTTCCGGTTGGACC

 LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu

 N
 S
 i
 I
 AGGCATTCAACAGGGCTGTCAAGAGTTACAGAATGCATCAGCAATTGAGAGCATTCTTA
 181 -----+-----+-----+-----+-----+-----+-----+ 240
 TCCGTAAGTTGTCCCGACAGTTCTCAAATGTCTACGTAGTCGTTAACTCTCGTAAGAAT

 AlaPheAsnArgAlaValLysSerLeuGlnAsnAlaSerAlaIleGluSerIleLeuLys

 AAAATCTCCTGCCATGTCTGCCCTGGCCACGGCCGACCCACGCGACATCCAATCCATA
 240 -----+-----+-----+-----+-----+-----+-----+ 300
 TTTTAGAGGACGGTACAGACGGGACCGGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTAT

 AsnLeuLeuProCysLeuProLeuAlaThrAlaAlaProThrArgHisProIleHisIle

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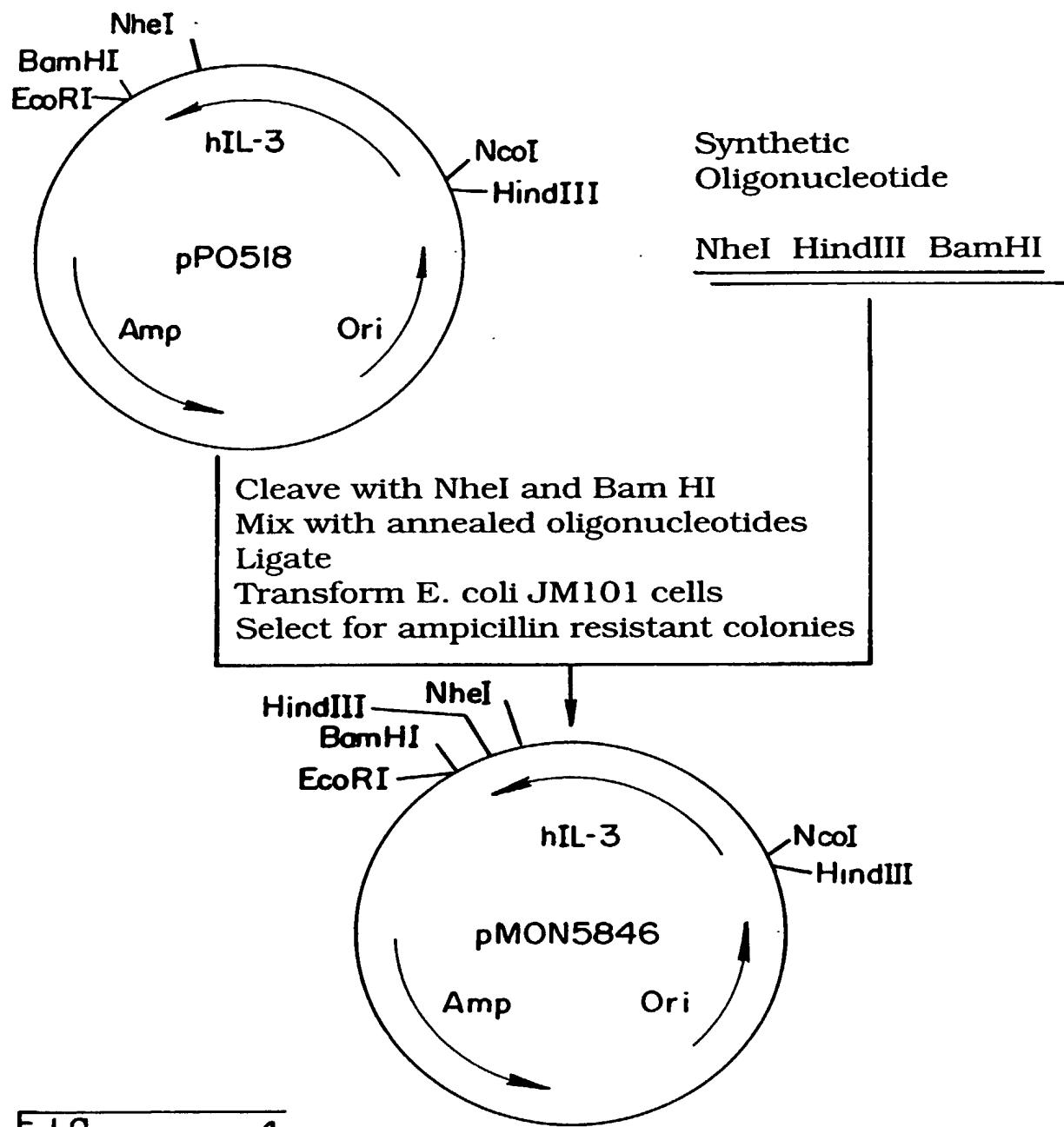
301 TCAAGGACGGTACTGGAATTCCGTCGAAACTGACCTCTATCTGAAAACCTGG
-----+-----+-----+-----+-----+-----+-----+ 360
AGTTCCCTGCCACTGACCTTACTTAAGGCAGCATTGACTGGAAGATAGACTTTGGAACC

LysAspGlyAspTrpAsnGluPheArgArgLysLeuThrPheTyrLeuLysThrLeuGlu

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361 AGAACGCGCAGGCTCAACAGACCACTCTGTCGCTAGCGATCTTTAATAAGCTT
-----+-----+-----+-----+-----+-----+ 414
TCTTGC CGTCCGAGTTGTCTGGTGAGACAGCGATCGCTAGAAAATTATCGAA

AsnAlaGlnAlaGlnGlnThrThrLeuSerLeuAlaIlePheEndEnd



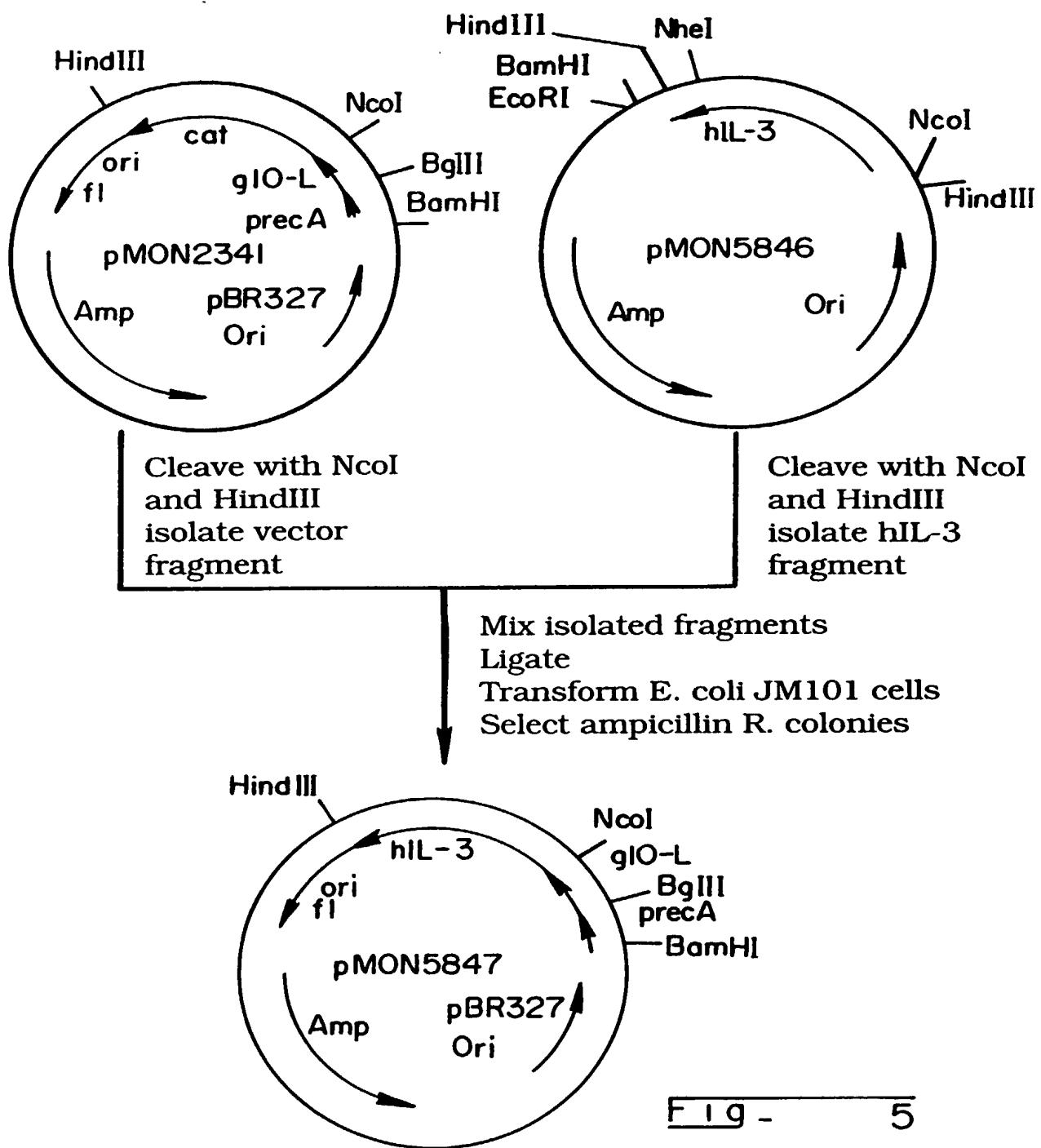
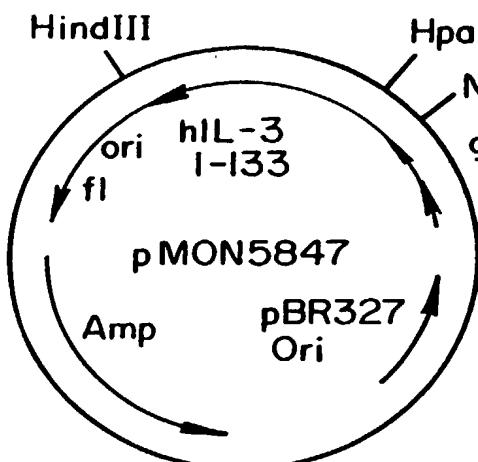
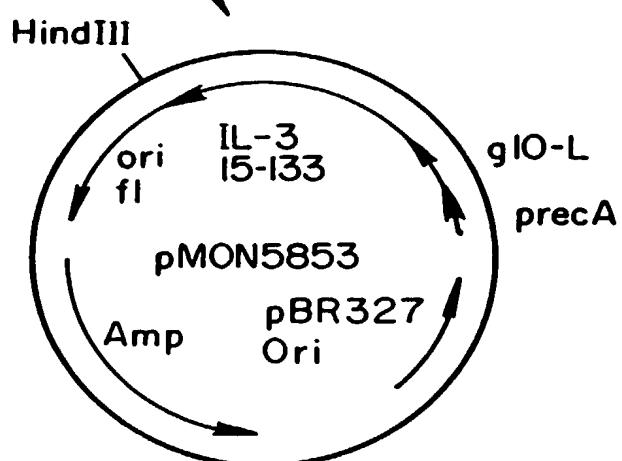
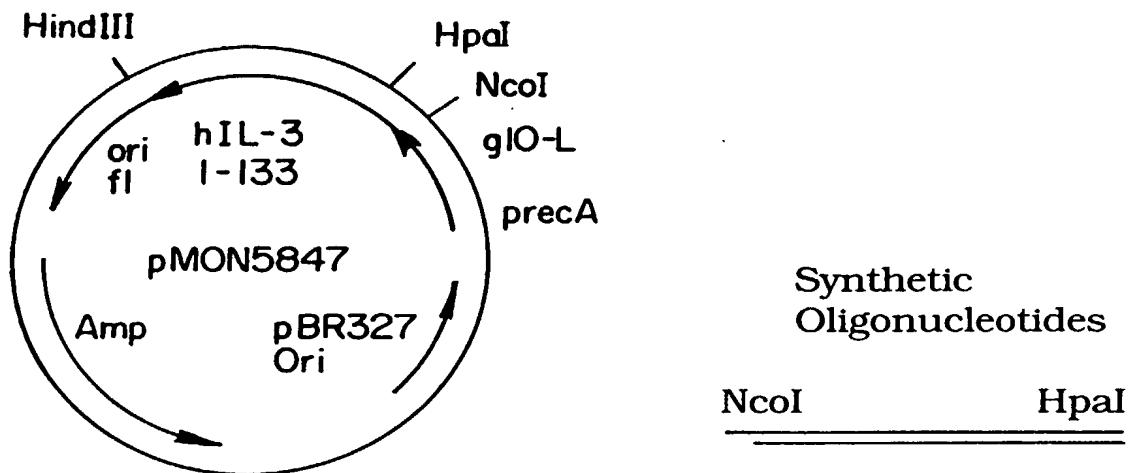


Fig - 5



Cleave with NcoI and HpaI.
Klenow fill NcoI end to
render it blunt.
Ligate the Blunt ends.
Transform E. coli JM101
to ampicillin resistance





Cleave with NcoI and HpaI.

Mix cleaved plasmid
with oligonucleotides.
Transform *E. coli* JM101
cells to ampicillin resistance.

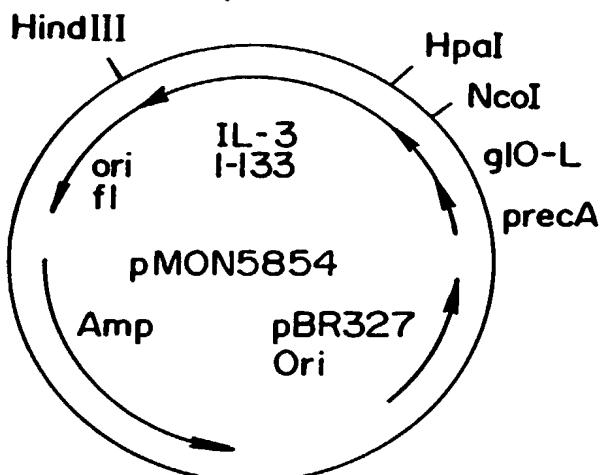


Fig - 7

1 ATGATGATTACTCTGGCAAACCTCTGGGGGTGTGCCCTCGCAGGGGGTAAATGTC
1 TACTACTAATGAGACGGGTTGAAGGAGACCGCCAACGGCAGGCTGCCCGCATACAGA
60

MetMetIleThrLeuArgLysLeuProLeuAlaValAlaAlaGlyValMetSer

N

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GCTCAGGCCATGGCTAACTGC
61 CGAGTCCGGTACCGATTGACG 81

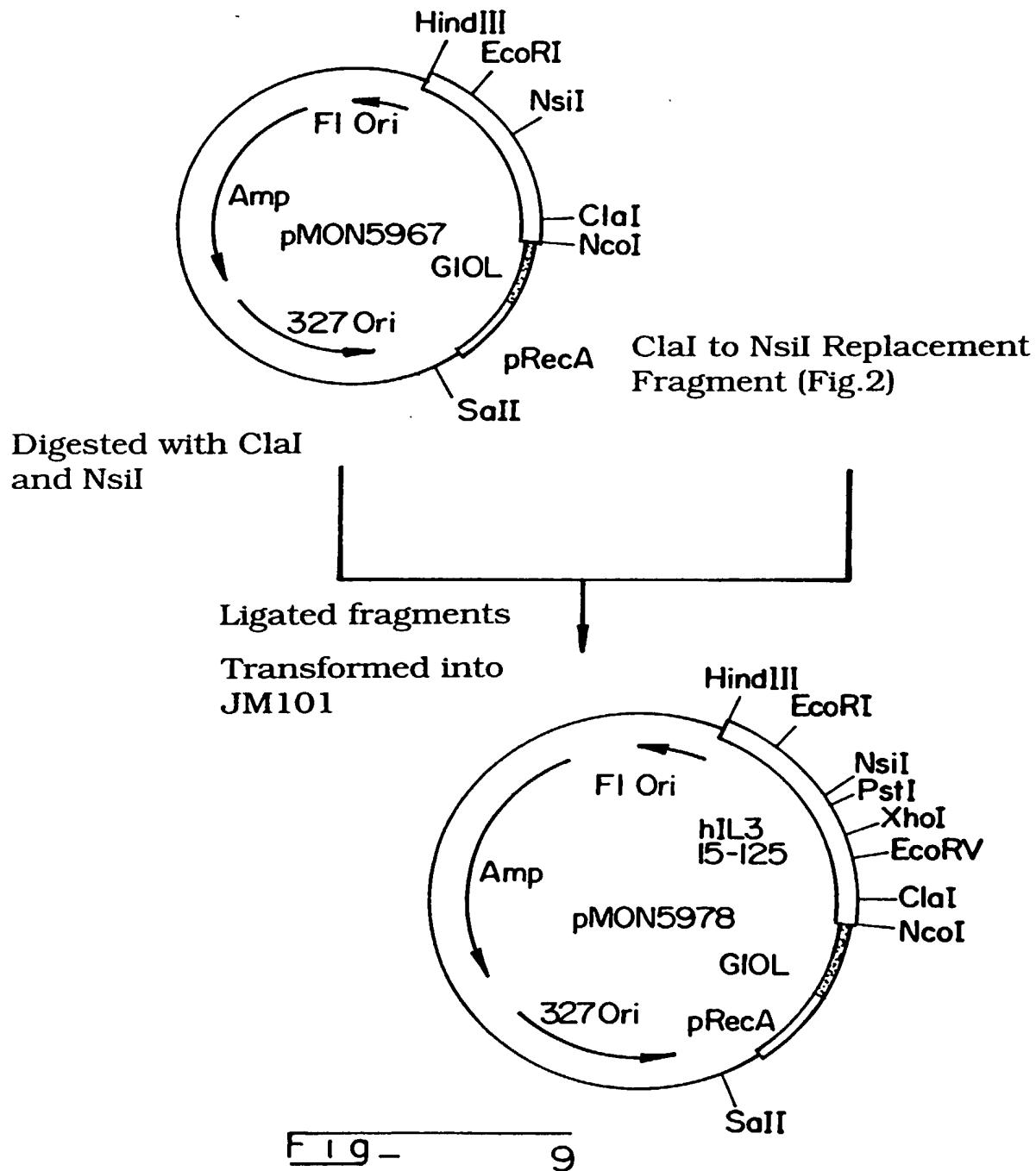
[SEQ ID NO: 149]
[SEQ ID NO: 150]

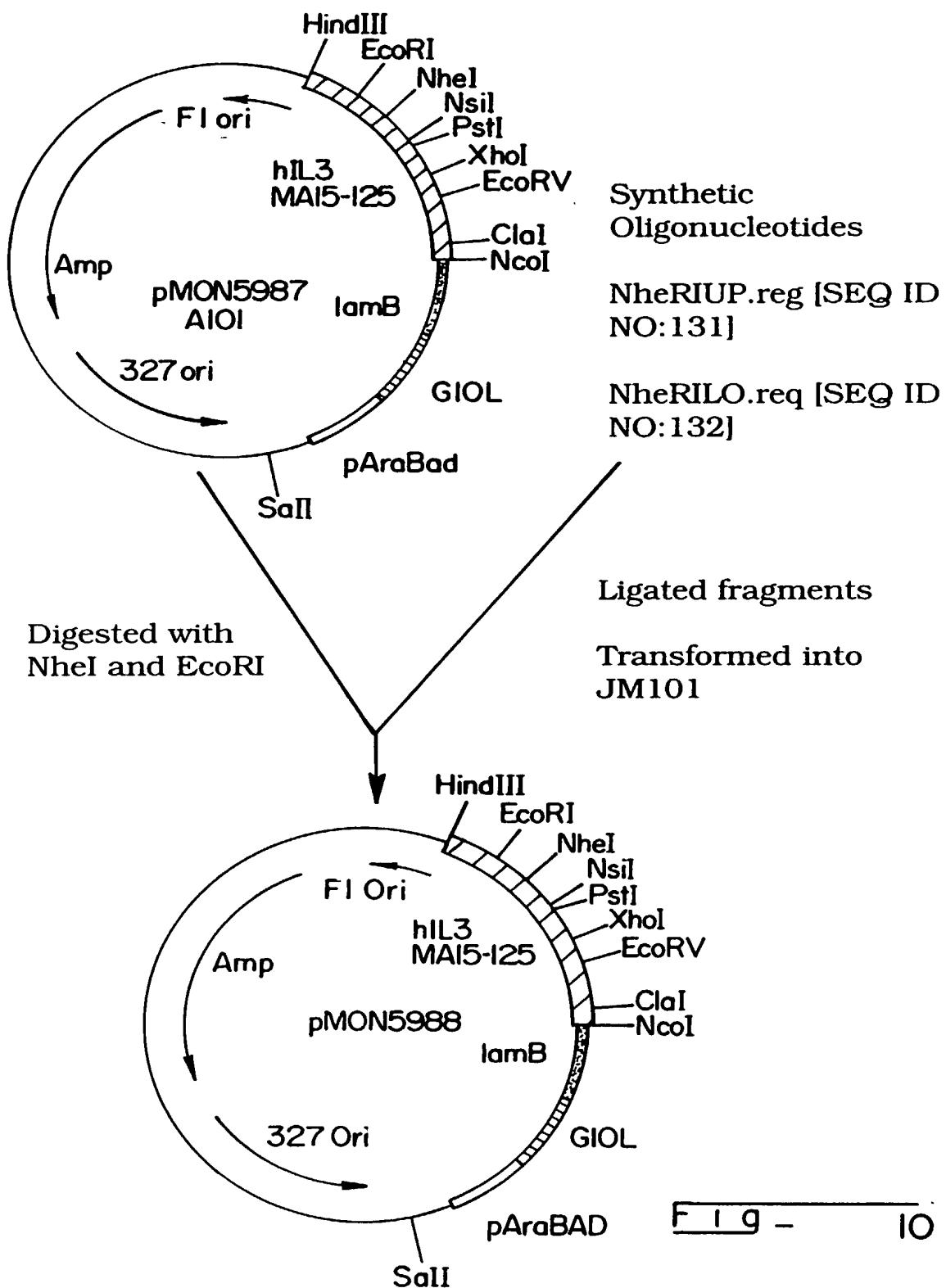
AlaGlnAlaMetAlaAsnCys

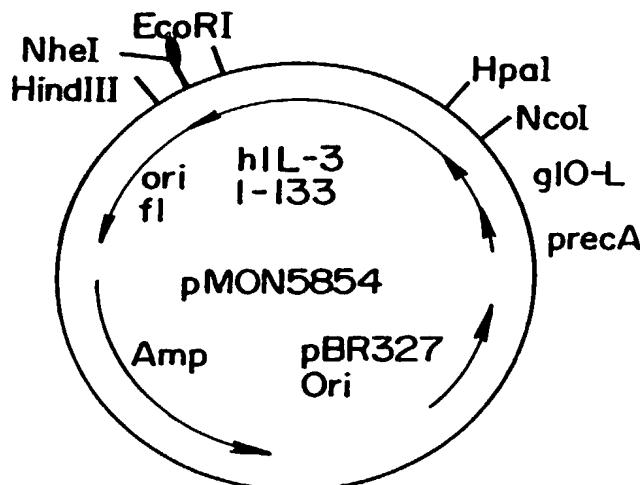
[SEQ ID NO: 14]

LamB Signal Peptide

F 1 0 - 8







Synthetic Oligonucleotides

EcoRI HindIII

DNA sequence terminate hIL-3 coding sequence after codon 125

Cleave with EcoRI and HindIII

Mix cleaved vector with oligonucleotides.
Transform E. coli JM101 cells to ampicillin resistance.

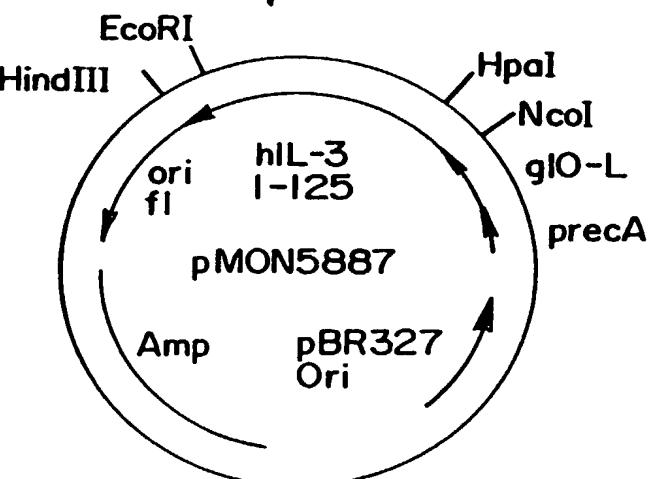


Fig - II

5' CATGGCTAACTGCTCTAACATGAT 3'
SEQ ID NO:151

3' CGATTGACGAGATTGTACTAGC 5'
SEQ ID NO:152

Annealed
Oligonucleotides

Ligated fragments

Transformed into
JM101

Digested with NcoI
and ClaI

Gel purified 4263 bp
fragment

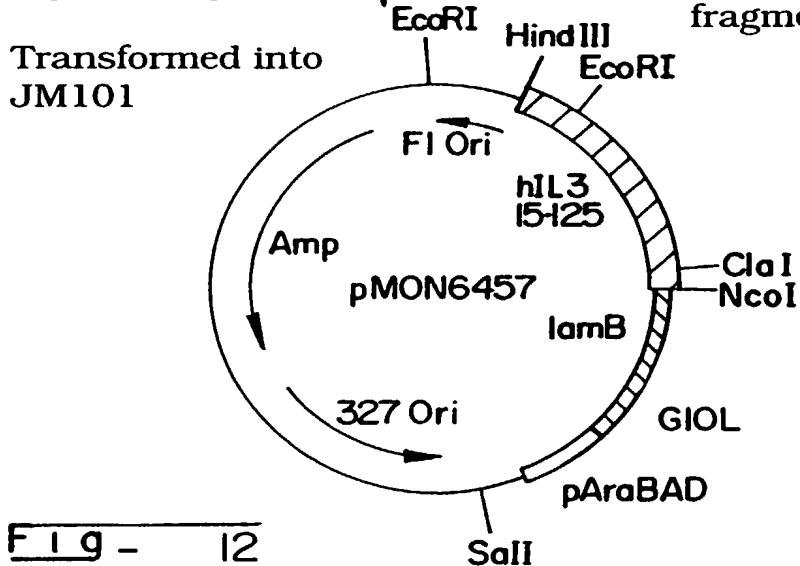
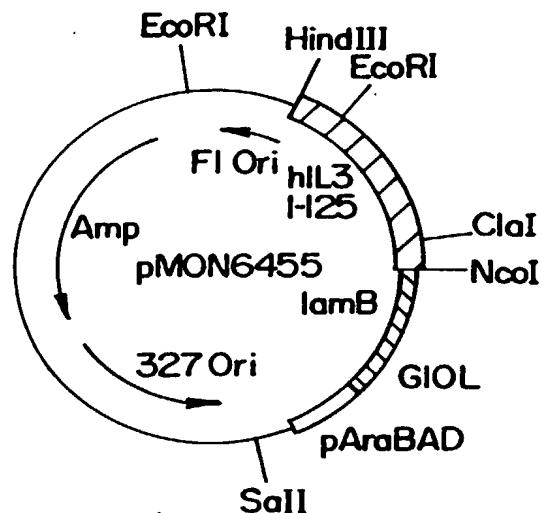
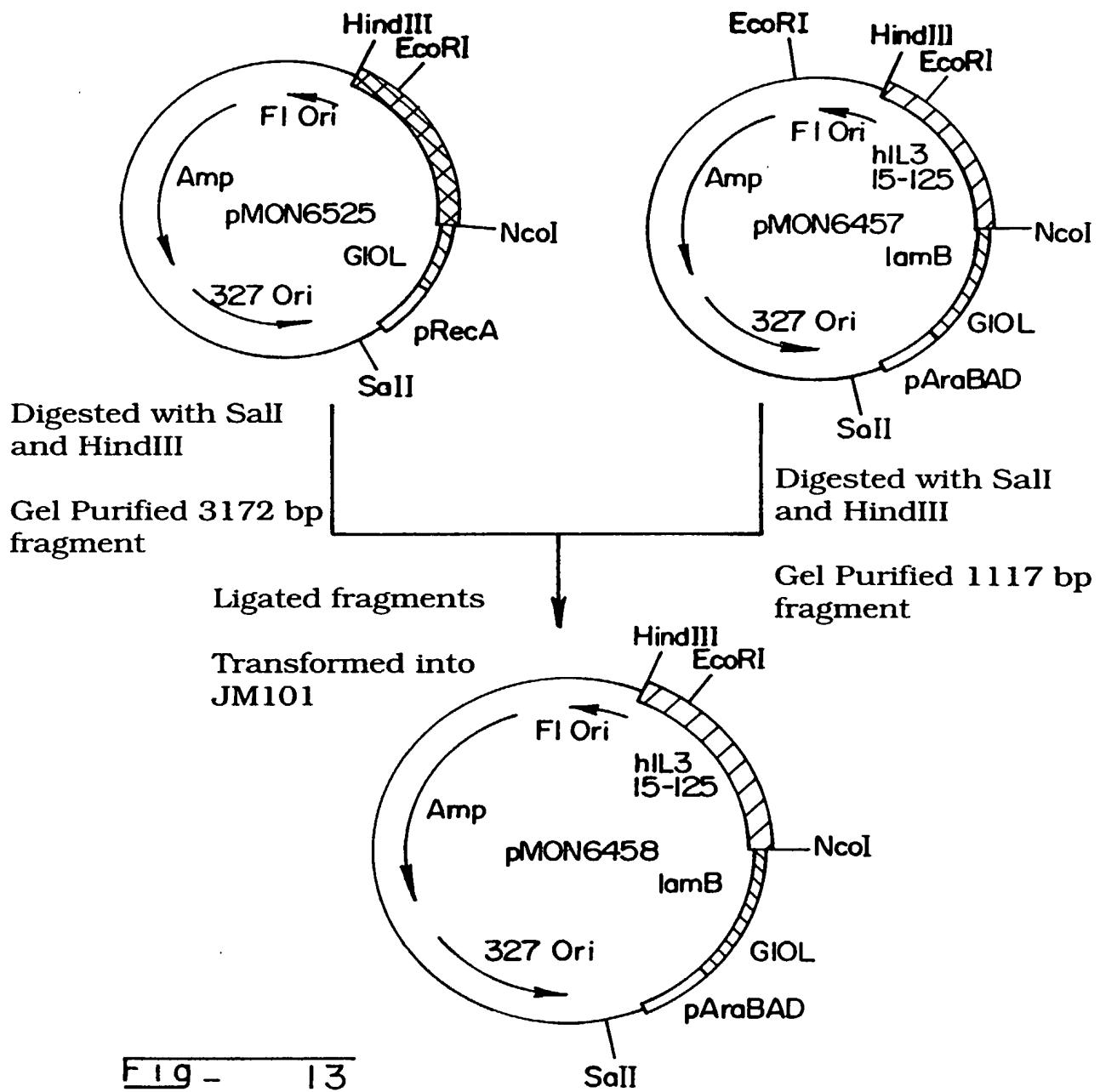
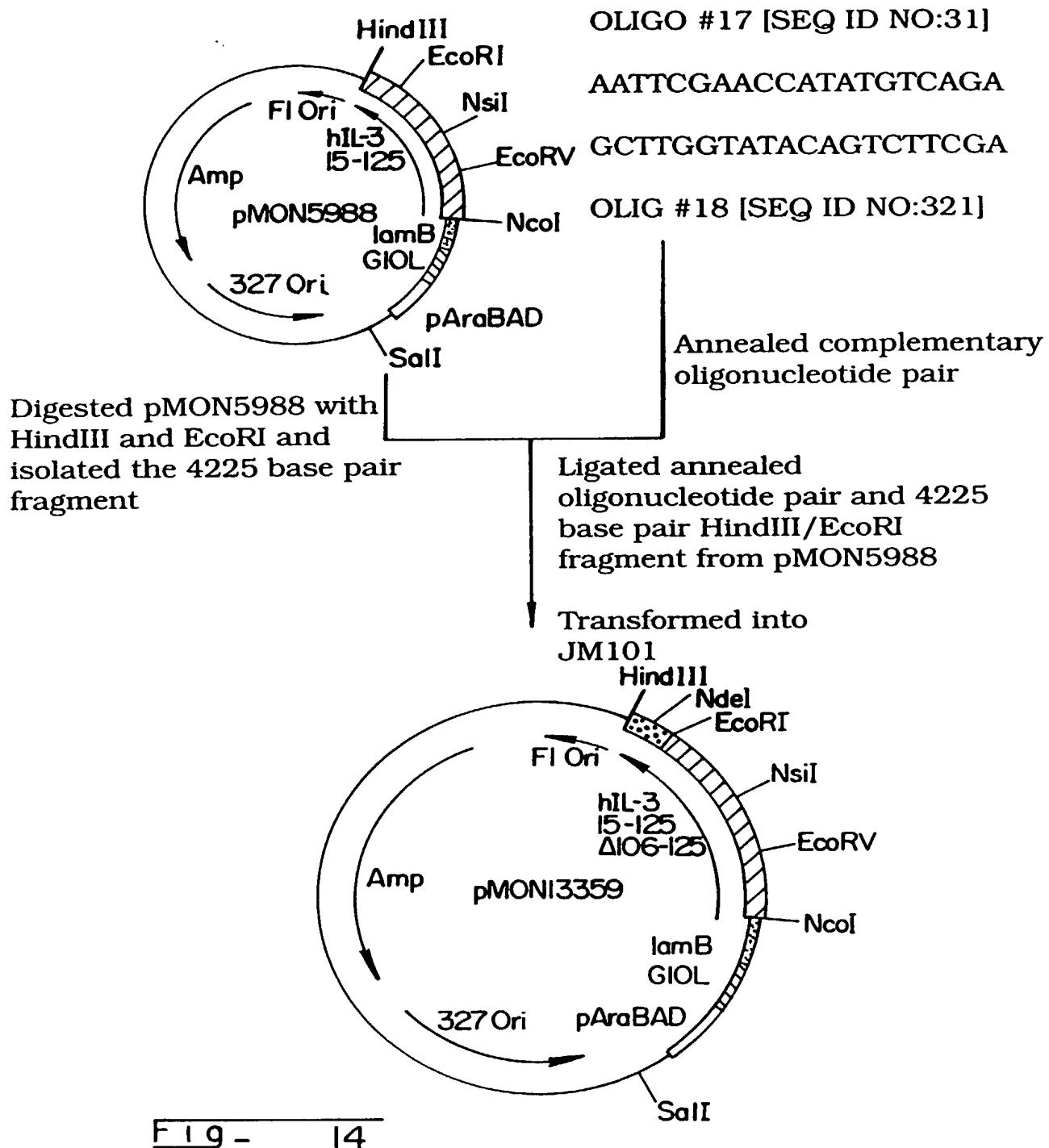


Fig - 12





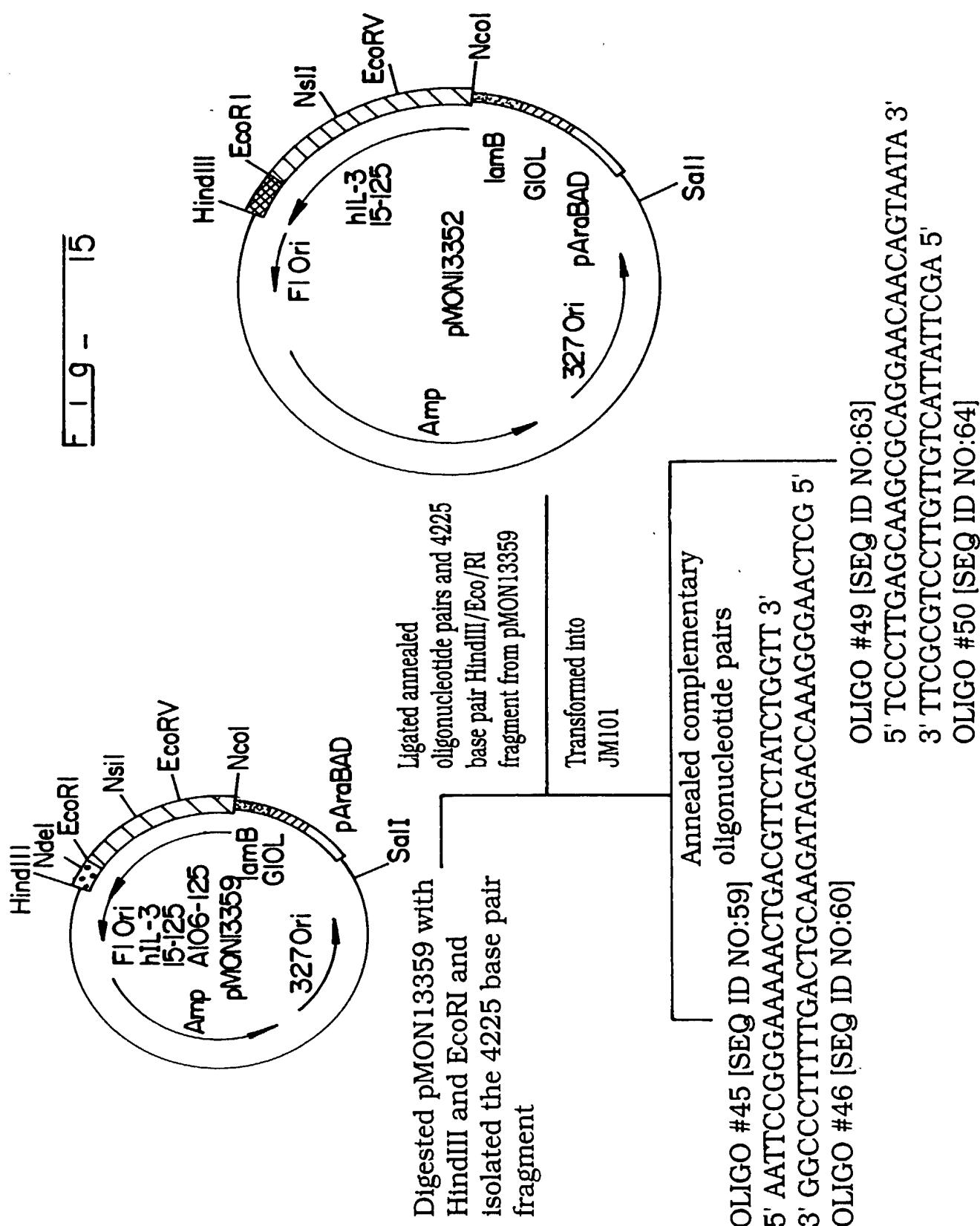
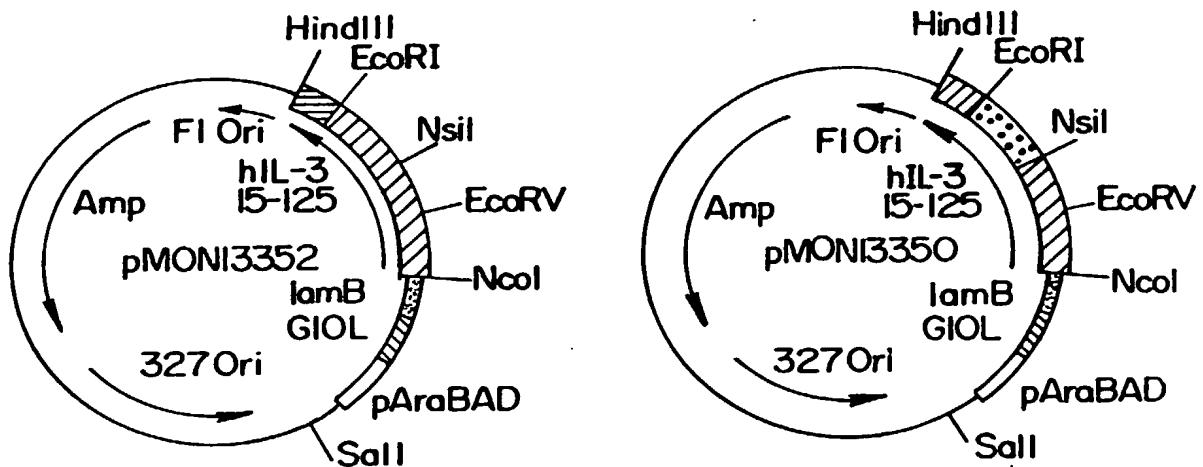


Fig - 16



Digested pMON13352 with
NsiI and EcoRI and
isolated the 4178 base pair
fragment

Digested pMON13350 with
NsiI and EcoRI and
isolated the 111 base pair
fragment

Ligated fragments

Transformed into
JM101

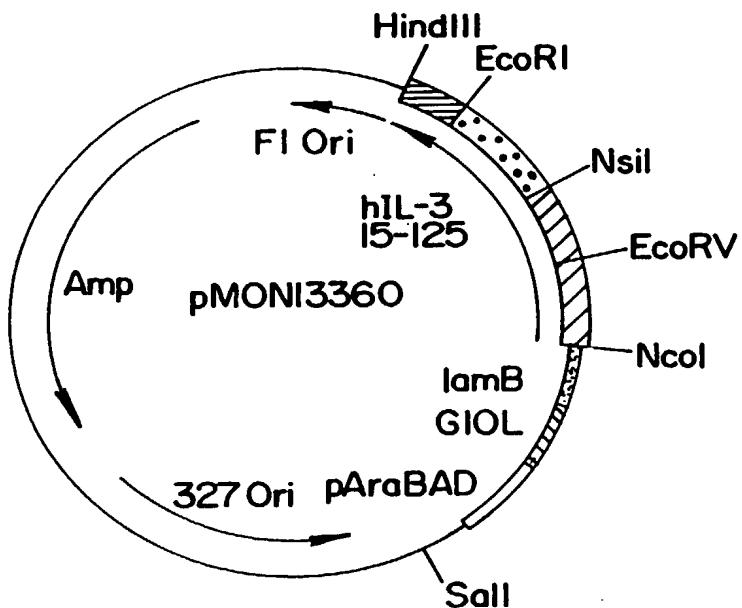
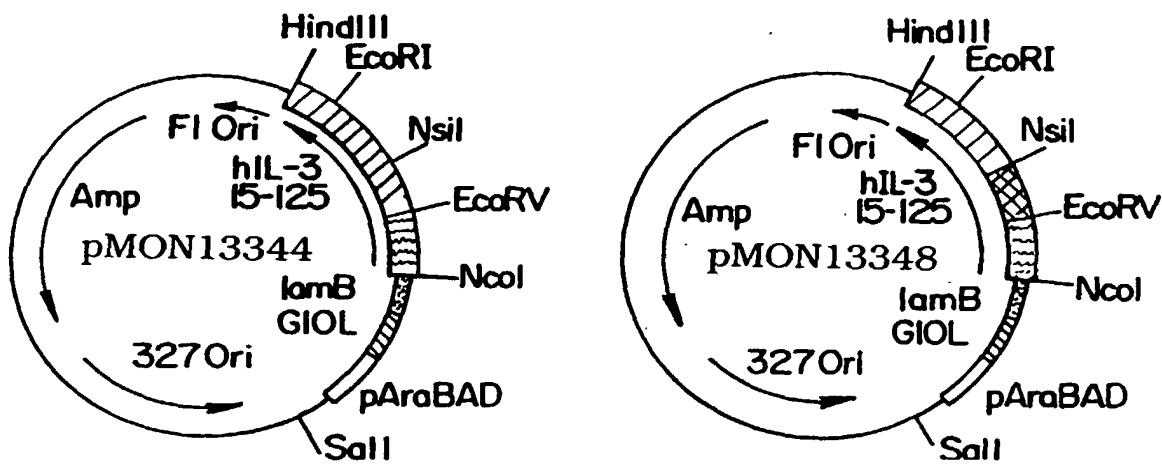


Fig - 17



Digested pMON13344 with Nsil and EcoRV and isolated the 4218 base pair fragment

Digested pMON13348 with Nsil and EcoRV and isolated the 71 base pair fragment

Ligated fragments

Transformed into JM101

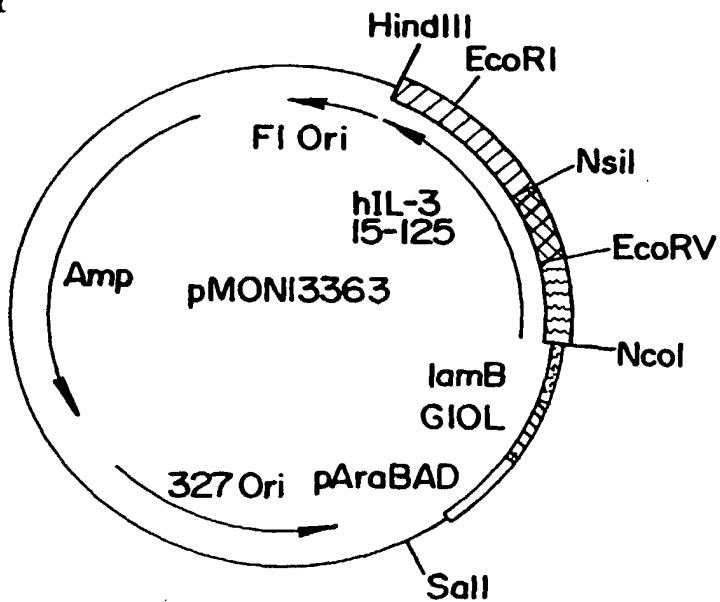
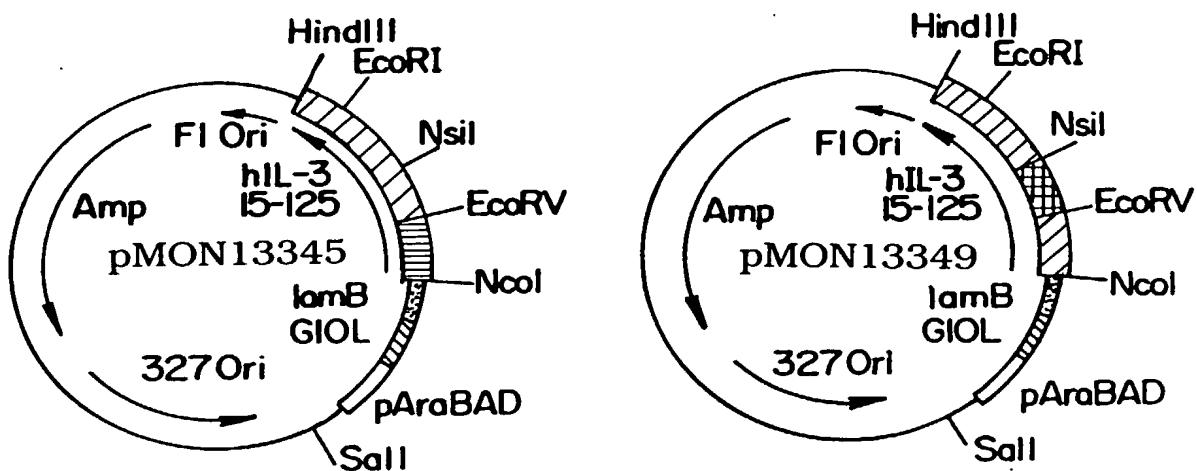


Fig - 18

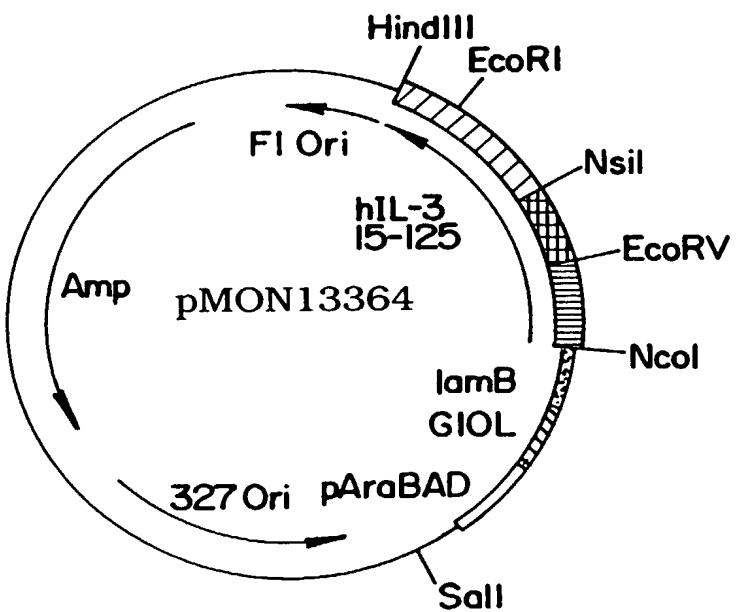


Digested pMON13345 with
NsiI and EcoRV and
isolated the 4218 base pair
fragment

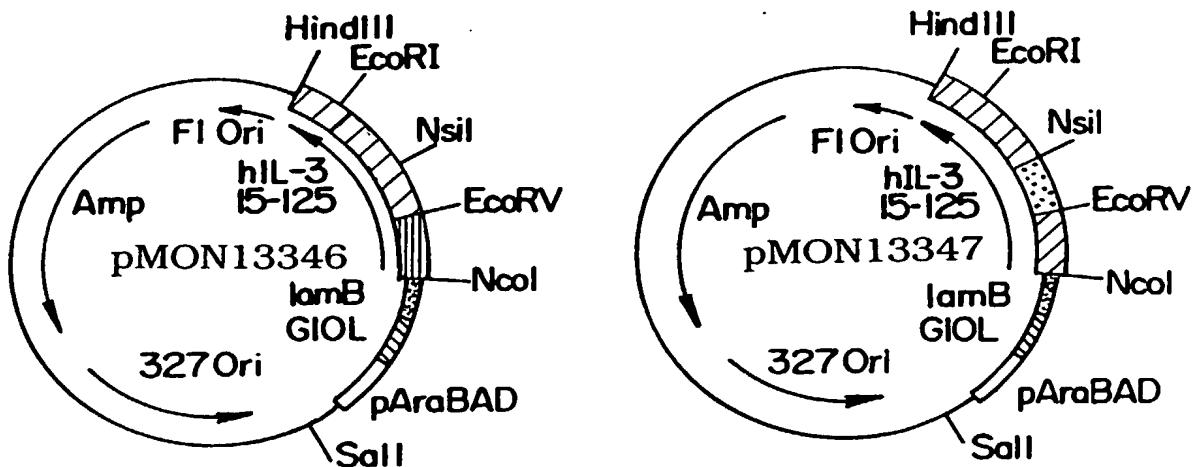
Digested pMON13349 with
NsiI and EcoRV and
isolated the 71 base pair
fragment

Ligated fragments

Transformed into
JM101



F 19 - 19



Digested pMON13346 with
NsiI and EcoRV and
isolated the 4218 base pair
fragment

Digested pMON13347 with
NsiI and EcoRV and
isolated the 71 base pair
fragment

Ligated fragments

Transformed into
JM101

